THE COUNCIL FOR TOBACCO RESEARCH–U.S.A., INC.

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(212) 421-8885

FEB 1 1974

Application for Research Grant
(Use extra pages as needed)

Date: Jan.3, 1974.

1. Principal Investigator (give title and degrees):

Sanford E. Leeds, M.D.
Asst. Clin. Professor of Surgery, University of California Medical School
Director, Experimental Surgery Laboratory, Mount Zion Hospital & Medical Center.

2. Institution & address:

Mount Zion Hospital and Medical Center
P. O. Box 7921
San Francisco, California 94120

3. Department(s) where research will be done or collaboration provided:

Experimental Surgery Laboratory

4. Short title of study:

Role of the pulmonary lymphatics in the absorption of inhaled particles and gases.

5. Proposed starting date: July 1, 1974

6. Estimated time to complete: 3 years

7. Brief description of specific research aims: The route of absorption and lung clearance of inhaled particles has been extensively studied (1,2,3,4). However, there are few studies of the role of the pulmonary lymphatics in the absorption process. The parenchyma of the lungs have an extensive network of lymphatics which drain lymph toward the hilar lymph nodes and thence into the venous circulation mainly via the right lymphatic duct (RD). The role of lymphatics in the transport of particles from the alveoli is not well understood because the lymphatic and respiratory systems are difficult to study both anatomically and physiologically. Morrow (5) in a recent review states "few experiments either have been designed or have attempted to quantify the role of pulmonic lymphatics in dust removal, and even fewer have tried to elucidate the mechanisms and pathways of lymphatic uptake and transport". The specific aims of the research to be described is to elucidate the role of the pulmonary lymphatics in the clearance of inhaled particles from the alveoli and interstitial tissue of the lung.

The specific objectives are threefold:

(1) To administer aerosols containing particles of different characteristics and to quantitate their appearance in pulmonary lymph proximally and distally to the hilar lymph nodes. The particles to be studied are in the range of 0.5 - 5.0 μm in size and include organic and inorganic, radioactive and non-radioactive, cytotoxic and inert and those having different degrees of solubility in body fluids.

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(2) To measure the rate of flow and composition (chemical and cellular) of pulmonary (right duct) lymph and thoracic duct lymph before and after the inhalation of various particles.

(3) To determine the number and types of cells in lymph from the right duct (RD) and thoracic duct (TD) after inhalation of various particles. Lymph from the RD is mainly pulmonary and cardiac in origin; lymph from the TD is mainly from the abdominal viscera and lower extremities.

In order to design the experiments the principal investigator, through the kindness of Dr. Ruy Lourenco, spent two half days with Dr. Peter Lee and Mr. Frank J. Hass in Dr. Lourenco's laboratory at the University of Illinois Medical School. A spinning disc generator was in use there to create monodispersed particles for administration to patients for lung scanning. Also, through the cooperation of Dr. R. O. McClellan and the staff of the Inhalation Toxicology Research Institute of the Lovelace Foundation of Albuquerque, New Mexico, three days were spent learning about the administration and quantitation of inhaled particles. The protocol to be presented was discussed with radiobiologists, aerosol physicists, chemists, physiologists, pathologists, etc., to whom we are indebted.

8. Brief statement of working hypothesis.

(1) The alveolar epithelium is relatively and selectively impermeable to many substances, but relatively permeable to others (6).

(2) Protein molecules are rapidly absorbed from the lungs of guinea pigs and dogs and appear in the blood (7,8,9,10,11). The role of the lymphatics in this absorption is not clear. Myer et al (12) suggest that ^{131}I-albumin is absorbed from the alveoli more rapidly by pulmonary capillaries than by pulmonary lymphatics. If so, this is different than the absorption of protein molecules from most tissues where the lymphatics are necessary for transfer of molecules from interstitial tissue to the venous circulation (13).

(3) Inhaled particles appear in interstitial tissue and hilar lymph nodes; therefore they must be absorbed by the alveoli and are presumed to be carried by parenchymal lymphatics to the hilar nodes. Inhaled particles may appear within the paraesophageal and paragastric (lesser curvature) lymph nodes of man (14). The para-aortic (abdominal) lymph nodes can receive lymph from the basal lobes of both lungs (15). Lauweryns (16) described juxta-alveolar lymph ducts which are presumed to conduct particles from the alveoli to the interstitial lymphatics. Whether the particles are free or engulfed by macrophages is not the concern of this investigation (17).

(4) Rate of transport of radioactive particles to hilar nodes and blood has been calculated by the use of lung models. The rate of lung node to lung concentration increases with time (1,2,3).

(5) Hard evidence of the presence of particles in pulmonary lymphatics is sparse. Lauweryns (18) demonstrated ferritin and carbon in interstitial lymphatics of the lung after endotracheal instillation. Morrow and Casarett (19)

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have demonstrated particles and particular aggregates in peribronchial lymphatics of dogs exposed to a PuO_2 aerosol. The permeability of lymphatics of the leg to substances of various molecular sizes has been studied by Myerson et al (20) by cannulation of afferent and efferent lymphatics of the popliteal node. Garlick and Renkin (21) describe the transport of large molecules from plasma to interstitial fluid and lymph in the paw of dogs in experiments in which lymphatics distal to the popliteal lymph node were cannulated.

(6) Particles may deposit in regional nodes, e.g., hilar nodes or may by-pass them depending on particle size and other less well defined factors (22,23). Deposition of particles can be variable as demonstrated by autoradiograms of adjacent lymph nodes after inhalation of plutonium-239 dioxide. One node contained many particles PuO_2 on the afferent side of the node, and only one or two particles were present in subcapsular sinusoids of the other(19).

(7) It is suggested that examination of pulmonary lymph and pulmonary vein blood after inhalation of particles may demonstrate the presence of particles which have by-passed hilar nodes. We have developed techniques for the collection of pulmonary and systemic lymph (24,25,26). By our improved method of collecting pulmonary lymph, the entire output of the RD is obtained for cellular and chemical examination and for the detection of particles.

(8) The ingestion of aerosol particles by the intestinal tract may be followed by the appearance of particles in thoracic duct lymph and blood. For this reason concomitant collection of TD lymph is necessary.

(9) Alterations in total and differential counts of cells in RD and TD lymph (26) after inhalation of particles are an index of the body's response to the effects of such inhalations. This may give new information on the effects of air pollution on the organism, particularly when particles are injurious.

(10). To summarize: Appearance of inhaled particles in pulmonary lymph may be dependent mainly on size (20). Small particles (less than 1 μm) are probably more likely to by-pass or traverse hilar nodes than larger particles. Solubility, cytotoxicity, chemical structure, i.e. organic or inorganic, electrical charge, etc. are other factors which may influence alveolar absorption and appearance of particles in lymph. By studying a series of particles of various sizes and chemical characteristics information on the role of the lymphatics in the absorption of particles may be obtained.

9. Details of experimental design and procedures.

(1) Collection of lymph from RD, TD and lobular lymphatic.
(2) Collection of blood: pulmonary venous (lobular) and peripheral blood.
(3) Administration of particles: endobronchial instillation, ultrasonic nebulized aerosol, and other methods of aerosol administration.

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(4) Measurement of concentration of particles in lymph and blood.
(5) Measurement of particles in hilar lymph nodes by light microscopy, electron microscopy and autoradiographs.
(6) Experiments in which hilar lymph nodes are excised.
(7) Cellular composition of lymph.

(1) Collection of lymph. The TD is cannulated in the neck near its junction with the venous system. Cream is given by stomach tube 2 hours prior to cannulation to facilitate visualization of the TD. The chest is not opened for either TD or RD cannulation.

The RD is cannulated in the neck by the improved method described by Leeds et al (24) and by Uhley et al (25). Evans blue dye (T-1824) is instilled into the right lung in order to aid in visualizing the leash of fine lymphatics whcih makes up the RD. A segment of the right external jugular and subclavian veins into which the pulmonary lymphatics drain is isolated by dissection and ligation, taking care to preserve the lymphatics. The segment of the external jugular vein is then cannulated and the entire pulmonary lymph drainage, except for a portion from the left upper lobe, is collected. This is an improvement over the method of Warren and Drinker (27) published in 1942, in which one small lymphatic was cannulated and only a small portion of the pulmonary flow obtained. Five hourly samples of lymph from the RD and TD are usually collected for measurement of rate of flow and for cellular and chemical analysis.

(2) Collecting blood. Peripheral venous blood is drawn from the femoral vein. Pulmonary venous blood is drawn from a lobular vein.

(3) Administration of particles. Solutes and suspensions of protein molecules such as ^{131}I-albumin can be administered by passing a 16 gauge catheter through a cuffed endotracheal tube or bronchoscope into the R and L mainstem bronchus and injection of the material with a syringe.

Aerosols containing non-radioactive or radioactive colloids or other particles are administered by an ultrasonic nebulizer. (Mistogen Electronic Nebulizer, Model EN 145, 2711 Adeline St., Oakland, California 94607). With this apparatus approximately 0.5 ml of solution can be converted into a dense aerosol mist per minute. The mean size of the aerosol particles is estimated to be 0.5 to 1.0 μm. A dual valve arrangement maintains flow in one direction in a closed system from nebulizer to subject to vented hood outlet. Radioactive solutions can be used in safety with this apparatus. About 10% of radioaerosol is deposited in the lung (28).

Should the results of experiments with this simple method warrant, a more sophisticated apparatus such as that described by Kanapilly et al (29) can be constructed. A spinning disc generator is a useful method to create monodispensed particles (30). Current techniques permit measurement of the size and concentration of particles delivered (31,32).

(4) Measurement of concentration of particles in lymph and blood. Non-radioactive particles are measured by light and electron microscopic methods. Particles can be counted in a hemocytometer in an aliquot of lymph.

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Radioactive particle concentrations in lymph or pulmonary venous blood are measured in a scintillation counting chamber.

The size of particles can be measured with a Porton graticule and stage micrometer (31).

Autoradiography techniques described by Leary (33), Evans (34) and Arnold (35) can be used to identify and measure radioactive particles with light microscopy. Electron microscopy can be used for radioactive and non-radioactive particles after they are collected on membrane filters and transferred to a carbon-coated electron microscope grid (36).

(5) Measurement of particles in hilar lymph nodes. Autoradiography of histologic sections as described by Evans (34), and Arnold (35) permits microscopical determination of the distribution of radioactive materials in the tissues. Particle size analysis is also achieved by autoradiography.

(6) Experiments in which hilar lymph nodes are excised. To determine the role of hilar lymph nodes in the removal of particles absorbed by the pulmonary alveoli, the experiments described above (Section 9 (3)) can be repeated in dogs in which the hilar and mediastinal lymph nodes have been excised. A sternal splitting incision gives access to the lymph nodes of the hilum of each lung, peritracheal and carinal areas. The hilar lymph nodes are excised and a period of 2 weeks or more allowed for regeneration of the pulmonary lymphatic pathways. Particles are administered after cannulation of the RD or lobular lymphatic and measurement of flow and composition of pulmonary lymph. Samples of pulmonary lymph are collected at 1/2 hourly intervals after inhalation of particles and the lymph examined for the presence of particles.

(7) All samples of RD and TD lymph are examined to determine total number of cells and differential counts of RBC and WBC (26).

10. Space and facilities available.

These include a modern experimental surgery laboratory, opened in 1963, with four operating tables each with piped-in suction, air and oxygen, special lights and Bird and Harvard respirators. Large preparation and work rooms adjoin. In addition, there is an individual room for the use of the investigators and technician personnel, which contains benches, desk, record files, book shelves, storage cupboards and drawers for special equipment. Major items of equipment which are available or permanently kept in the laboratory are: 6-channel Electronic for Medicine recording oscillograph, portable x-ray machine, electron microscope, blood gas analysis apparatus, centrifuge, Mistogen electronic nebulizer, fume hood and a Zeiss operating microscope equipped with camera and Strobe light. In addition, the facilities of the Division of Nuclear Medicine are available for determination of radioactivity in blood and lymph and for scintillation scans and radioautographs of the lung. The facilities of the Immunology Research Laboratory are available for cell counts of lymph, hematologic and immunologic (i.e. electrophoresis) studies. The facilities of the Pulmonary Laboratory are available for administration

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of aerosol particles and gases. The personnel of these three sections collaborate closely with the principal investigator and his associates in a team effort. All the above facilities are under one roof, the Mount Zion Hospital and Medical Center.

11. Additional facilities required: None

12. Biographical sketches of investigators and other professional personnel: See Appendix I., pp.12-19.

13. Publications: See Appendix II., p.20.

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14. First year budget:

A. Salaries (give names or state "to be recruited") Professional (give % time of investigator(s) even if no salary requested)	% time	Amount
Sanford E. Leeds, M.D., Principal Investigator	40	12,000
Philip E. Mann, M.D., Co-Investigator	10	2,500
Herman N. Uhley, M.D.	5	0
Theodore N. Finley, M.D.	5	0
Kenneth R. McCormack, M.D.	5	0
Jay Gershow, M.D.	5	0
Technical		
Technician II (Aerosol)	100	12,000
Fringe benefits 15%		4,194
Sub-Total for A		30,694
B. Consumable supplies (by major categories)		
Dogs at $25.00 each	2,000	
Surgical supplies, endotracheal tubes, plastic tubes, etc.	500	
Radioactive isotopes, chemicals	1,000	
Oxygen, miscellaneous gases	200	
Sub-Total for B		3,700
C. Other expenses (itemize)		
Publication	300	
Photography, Medical Artist	75	
Lab. tests, Nuclear Medicine Lab. costs	500	
Travel to meetings on aerosols, air pollution, etc.	500	
Sub-Total for C		1,375
Running Total of A + B + C		35,769
D. Permanent equipment (itemize)		
Parts to build aerosol administration apparatus (aerosol generator, nebulizer, heating column, filters, etc., cascade impactor, electrostatic precipitator, etc.	3,000	
Sub-Total for D		3,000
E. Indirect costs (15% of A + B + C)		5,365
Total request		44,134

15. Estimated future requirements:

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Indirect Costs	Total
Year 2	32,382	3,700	1,375	2,000	5,618	45,075
Year 3	34,163	3,700	1,375	2,000	5,885	47,123

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16. Other sources of financial support:
List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Relation of lymphatics to cardiopulmonary function.	Department of Health, Education and Welfare HL 3180-16 to 3180-20	276,000	Dec.1, 1973 - Nov.30,1977.
		2nd year 53,093	Dec.1, 1973 - Nov.30,1974.

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
None			

is understood that the investigator and institutional officers in applying for a grant have read and accept ne Council's "Statement of Policy Containing Conditions nd Terms Under Which Project Grants Are Made."

Principal investigator

Typed Name Sanford E. Leeds, M.D.

Signature Sanford E. Leeds Date 1/24/74

Telephone 415 (Area Code) 567-0950 (Number) (Extension)

hecks payable to
Mount Zion Hospital and Medical Center

Mailing address for checks
Mr. George A. Thompson, Controller
~~Mount Zion Hospital and Medical Center~~
P. O. Box 7921, San Francisco, Cal. 94120

Responsible officer of institution

Typed Name Jay Okun Yedvab

Title Executive Director

Signature Jay Okun Yedvab Date 1/25/74

Telephone 415 (Area Code) 567-6600 (Number) 201 (Extension)

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